Perspectives on Improvement of Reproduction in Cattle during Heat Stress in a Future Japan

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Running Head: NEW PERSPECTIVES ON HEAT STRESS IN CATTLE

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ABSTRACT

Heat stress (HS) causes hyperthermia, and at its most severe form, can lead to death. More commonly, HS reduces feed intake, milk yield, growth rate and reproductive function in many mammals and birds, including the important cattle breeds in Japan. Rectal temperatures greater than 39.0°C and respiration rates greater than 60 per minute indicate cows are undergoing HS sufficient to affect milk yield and fertility. Heat stress compromises oocyte quality and embryonic development, reduces expression of estrus and changes secretion of several reproductive hormones. One of the most effective ways to reduce the magnitude of HS is embryo transfer, which bypasses the inhibitory effects of HS on the oocyte and early embryo. It may also be possible to select for genetic resistance to HS. Cooling can also improve reproductive performance in cows and heifers, and probably, the most effective cooling systems currently in use are those that couple evaporative cooling with tunnel ventilation or cross ventilation. Its effect to improve reproductive performance in Japan remains to be evaluated.

Key words: *heat stress, embryo transfer, cattle reproduction, thermoregulation.*

INTRODUCTION

Heat stress (HS) can be defined as the forces external to the animal that act to displace body temperature from set-point temperature (Hansen 2009). Body temperature is closely regulated by matching heat production with heat loss to the environment via conduction, convection, radiation and evaporation. At its most severe, HS induces heat stroke and death in domestic animals. It also reduces feed intake, productivity and reproduction (Collier *et al.* 2006; Hansen 2009). An example of a cow exposed to HS is shown in Figure 1.

Japan has experienced unusual summer weather in the past two decades, most notably in 1994 and 2010. In 1994, 4,258 dairy cows were killed by HS, and financial losses totaled 127 million yen. The Ministry of Agriculture, Forestry and Fisheries reported that the 2010 summer heat wave of 1 July to 15 August killed 959 dairy cows, 235 beef cattle, 657 pigs, and 425,000 chickens. A particularly serious loss occurred in Tohoku region located in the northeastern portion of Honshu island. The climate of Tohoku is cooler than in other parts of Honshu and the severity of the HS problem might have been due to a failure of animals to be sufficiently adapted to HS or by failure of farmers to take effective countermeasures. The importance of HS for dairy production can be recognized by not only considering deaths caused by HS but by the loss of milk production and reproduction (Kadokawa 2011).

Heat stress is not confined to dairy cows in hot climates. Indeed, hyperthermia occurs in lactating dairy cows at temperatures as low as 25 to 28°C (Berman *et al.* 1985; Sartori *et al.* 2002) and decreased reproductive function during the summer has been reported in regions with temperate climates (Udomprasert & Williamson 1987; Sartori *et al.* 2002; Ambrose *et al.* 2006). The summer suppression of production and reproduction in Holsteins occurs even in Hokkaido (Kadokawa 2007), which is cool and one of the most important dairy farming area in Japan. Lactating cows are more sensitive to HS than non-lactating heifers (Badinga *et al.* 1985; Sartori *et al.* 2002) because lactating cows consume more feed and produce more heat than nonlactating heifers (Berman 2005). Nonetheless, HS can affect non-lactating cows. Recently, Sakatani *et al.* (2011) reported that summer heat affects estrous behavior and reproductive function in Japanese Black beef cattle.

The inhibitory effects of HS on production and fertility are likely to increase (Hansen 2007), given that increased heat generation due to improvements in milk production can make it more difficult to regulate body temperature during HS (Berman *et al.* 1985; Berman 2005). Global climate change will also exacerbate the problem of HS. Therefore, strategies that mitigate the negative effects of HS on reproductive function are likely to become essential for continued improvement in reproductive efficiency of dairy and beef cows.

This review aims to briefly describe mechanisms by which HS compromises reproduction and describe countermeasures that can be taken at the animal and facility level to reduce the impact of HS.

EVALUATION INDICES OF THE MAGNITUDE OF HS IN SUMMER

The magnitude of HS is caused by the combined effects of dry bulb temperature (T_{db}), humidity, solar radiation, and wind speed. Japan has four distinct seasons, autumn, winter, spring, and summer but a variety of climates because of the wide range of latitude (from 25 degN to 45 degN) and longitude (from 122 degE to 145 degE) encompassing the country. Two primary factors influence Japan's climate: its location near the Asian continent and the existence of major oceanic currents.

In general, Japan is a rainy country and the climate from June to September is marked by hot, wet weather brought by tropical airflows from the Pacific Ocean and Southeast Asia. There is a marked rainy season, Tsuyu, that begins in early June and continues for about a month. It is followed by a hot and sticky summer, with T_{db} as high as 40°C and relative humidity as high as 92 %. Five or six typhoons pass over or near Japan every year from early August to early September. About 70 to 80 percent of the 100-200 cm annual precipitation falls in the period between June and September.

Given the wide variety of climates in Japan, it would be useful to have an index estimating the magnitude of HS to aid farmers. Many temperature-humidity indices (THI) have been developed but these are only slightly better than T_{db} alone in predicting rectal temperature during HS (Dikman & Hansen 2009). In Florida USA, a T_{db} of 29.7°C was associated with an average rectal temperature of 39°C (mild hyperthermia), and a T_{db} of 31.4°C was associated with an average rectal temperature of 39.5°C (Dikmen & Hansen 2009). Using dairy cows in south-western Japan (Kumamoto prefecture), Tani *et al.* (2010) reported that pregnant cows had lower rectal temperature than non-pregnant cow at Day 7 (38.7°C vs. 39.4°C, $P<0.05$) or 8 (38.8°C vs. 39.1°C, $P<0.05$) after artificial insemination (AI). Probably the best method for assessing HS is to measure rectal temperatures and respiration rates during the afternoon in a few sentinel cows. Rectal temperatures greater than 39.0°C, and respiration rates greater than 60 per minute indicate cows are undergoing HS sufficient to affect milk yield and fertility. This recommendation is based on the observation that milk yield declined when rectal temperatures reached (39°C) (Zimbelman *et al.* 2009) and that conception rate declined 6.9-12.8% for each 0.5°C increase in uterine temperature above the mean temperature of 38.3-38.6°C (Gwazdauskas *et al.* 1973). Note that rectal temperature is about 0.2°C lower than uterine temperature (Gwazdauskas *et al.* 1973).

EFFECTS OF HEAT STRESS ON CONCEPTION RATES

In many areas of the world, conception rates decrease dramatically in dairy cows in the summer compared with other seasons (Zeron *et al.* 2001; Sartori *et al.* 2002;

Garcia-Ispierto *et al.* 2007; Huang *et al.* 2008; Flamenbaum & Galon 2010). Summer HS can also decrease the conception rates of beef cows (Azzam *et al.* 1989) including Japanese Black cows in southern part of Japan (Ogawa *et al.* 1978).

There are many causes for low conception rate during the summer including reduced oocyte quality (Gendelman *et al.* 2010; Sherab-El-Deen *et al.* 2010), failure of fertilization (Sartori *et al.* 2002), reduced embryonic development (Ealy *et al.* 1993; Sartori *et al.* 2002), and altered secretion of various hormones.

Oocytes collected from Holstein cows during summer possess decreased ability to develop to the blastocyst stage after in vitro fertilization (IVF) when compared with oocytes collected during winter (Rocha *et al.* 1998; Al-Katanani *et al.* 2002a, Gendelman *et al.* 2010). Lower fertility of repeat-breeder Holstein cows is associated with poor oocyte quality and this negative effect is enhanced during HS (Ferreira *et al.* 2011). The mechanism by which HS during oogenesis compromises oocyte function is likely to involve alterations in follicular function. Heat stress causes deviations in follicular growth by increasing numbers of small and medium follicles (Roth *et al.* 2000) and reducing the ability of the dominant follicle to exert dominance (Wolfenson *et al.* 1995).

Heat stress can alter steroid secretion in goat and dairy cows (Ozawa *et al.* 2005; Wilson *et al.* 1988). Plasma concentrations of progesterone and LH can decrease during the summer in dairy cows (Wolfenson *et al.* 2000). In the goat, HS decreases ovarian LH receptors (Ozawa *et al.* 2005), and HS reduces follicular responsiveness to LH (Kanai *et al.* 1995). Heat stress reduces circulating concentrations of inhibin and increases FSH secretion (Roth *et al.* 2000). Hyperthermia also affects cellular function in various tissues of the female reproductive tract including the follicle, oocyte and the embryo (Wolfenson *et al.* 2000; Hansen 2009).

EFFECTS OF HEAT STRESS ON ESTRUS DETECTION

Detection of estrus becomes difficult under HS, because dairy cows have reduced signs and duration of estrus during summer compared to winter (Monty & Wolff 1974; Wolff & Monty 1974; Piccione *et al.* 2003). Recently, Sakatani *et al.* (2011) reported that walking activity during estrus was less in the summer compared to the winter in Japanese Black cattle. Sakatani *et al.* (2011) also reported that duration of the estrous cycle was longer in summer (23.4 days, $P<0.05$) than winter (21.5 days) in this breed.

One possible reason for the reduced estrous behavior and extended estrous cycle in summer is a reduction in concentrations of estradiol-17β (Wilson *et al.* 1998). Pulsatile LH secretion, which is important to stimulate estradiol-17β secretion, is suppressed in dairy cows during summer (Gilad *et al.* 1993). Female goats under HS have a suppressed LH surge response to gonadotropin-releasing hormone (GnRH) (Kanai *et al.* 1995). A reduction in the LH preovulatory surge could conceivably lead to delayed ovulation (Siddiqui *et al.* 2010).

Recently, suppression of pulsatile LH release and the preovulatory LH surge reported previously in hot climates (Wise *et al.* 1988; Gilad *et al.* 1993; Chebel *et al.* 2004) has been also reported to occur in Hokkaido in northern Japan (Kadokawa 2007). Prepubertal heifers received a GnRH injection in May, July or November, and serial blood samples were collected to analyze the LH response curve. There were no significant differences in basal or peak LH concentrations or the area under the LH response curve among the three groups. However, the July group experienced the LH peak sooner ($P < 0.05$) than the May group. Therefore, HS may change facets of the mechanism controlling LH release in response to GnRH.

One effective way to bypass effects of HS on detection of estrus is to implement timed artificial insemination programs in which various drugs such as GnRH, prostaglandin $F_{2\alpha}$ and progesterone are used to program ovulation (Hansen & Arechiga 1999).

EMBRYO TRANSFER TO OVERCOME HEAT STRESS

Reductions in estrus detection may be overcome by the use of ovulation synchronization protocols like OvSynch, but preventing infertility caused by HS has been more difficult. The reproductive performance of Holstein cows compromised by HS can be improved by embryo transfer (ET). Early stage embryos are more susceptible to HS than the later stage embryos (blastocysts) (Hansen 2009). Embryo transfer improves pregnancy rates in summer because embryos are transferred after the time at which they are most sensitive to HS. Compared to AI, pregnancy rate for cows exposed to HS has been improved by transfer of either unfrozen embryos produced by superovulation (Ambrose *et al.* 1999; Demetrio *et al.* 2006) or in vitro production (IVP) (Al-Katanani *et al.* 2002b; Stewart *et al.* 2011) or by transfer of cryopreserved embryos produced by superovulation (Ambrose *et al.* 1999). In Kumamoto Prefecture where is very hot in summer, transfer of an IVP Japanese Black embryo following AI of Holstein semen in dairy cows resulted in greater pregnancy rate than for pregnancy rate after conventional AI (Tani *et al.* 2010). On the other hand, embryo transfer has not improved pregnancy rate when cryopreserved embryos produced by in vitro were transferred (Al-Katanani *et al.* 2002b; Stewart *et al.* 2011). The problem of poor estrus detection during HS has been overcome by the development of timed ET procedures based on the use of ovulation synchronization regimens such as OvSynch developed for timed AI (Al-Katanani *et al.* 2002b; Stewart *et al.* 2011).

In a recent study, transfer of fresh IVP embryos using sex-sorted semen to lactating dairy cows during summer increased the percentage of cows that established pregnancy and that gave birth to a live heifer compared with cows bred by AI with conventional semen (Stewart *et al.* 2011).

Despite the effectiveness of ET during the summer, use of this approach commercially has been limited. The high costs of embryo production by superovulation and transvaginal ovum pickup may be overcome through the use of abattoir-derived oocytes in conjunction with IVP. However, decreased survival following cryopreservation (Al-Katanani *et al.* 2002b; Stewart *et al.* 2011) limits the widespread application of IVP embryos in the commercial dairy industry. Improvements in the culture media to produce embryos offers opportunities for producing embryos in vitro with high potential for surviving cryopreservation and for establishment of pregnancy, although IVP systems are still not optimal (Block *et al.* 2010; Stewart *et al.* 2011).

Insulin-like growth factor-1 (IGF1) can improve resistance of day 5 preimplantation embryos to heat shock but not two-cell embryos (Jousan & Hansen 2007; Bonilla *et al.* 2011). Treatment of cultured embryos with IGF1 improves embryo survival after transfer into heat-stressed recipients but not after transfer into recipients not exposed to HS (Block & Hansen 2007; Loureiro *et al.* 2009).

NUTRITIONAL MANAGEMENT

An effective nutritional strategy for reducing effects of HS on reproduction has not yet been developed. One approach has been to administer antioxidants predicated on the idea that free radicals generated as a result of hyperthermia contribute to increased embryonic mortality (Hansen 2007). However, fertility of heat-stressed cows have not been improved by antioxidant treatments, including vitamin E (Paula-Lopes *et al.* 2003), selenium (Paula-Lopes *et al.* 2003), and short-term treatment with β-carotene (Arechiga *et al.* 1998a). However, long-term feeding of β-carotene (at least 90 days) did improve the proportion of cows pregnant by 120 days postpartum (Arechiga *et al.* 1998b). Other nutritional approaches, such as feeding polyunsaturated fatty acids (Bilby *et al.* 2006) and yeast cultures (Bruno *et al.* 2009), did not cause a significant effect on fertility in dairy cows.

Feeding encapsulated niacin increased evaporative heat loss during peak thermal load and was associated with a small but detectable reduction in rectal and vaginal temperatures in lactating dairy cows experiencing mild HS (Zimbelman *et al.* 2010). Effects of feeding encapsulated niacin on fertility under HS remains to be evaluated.

GENETIC IMPROVEMENT

There is sufficient diversity among beef breeds in thermotolerance to allow utilization of tropically-adapted breeds in some countries (Hansen 2009). However, such a strategy is not feasible for Japan, because Japanese Black cattle have advantages in terms of meat quality that cannot be found in other breeds. In dairy cattle, the differences in milk yield between tropically-adapted breeds and breeds from Northern Europe that have been selected for milk yield are so great that it is not economically feasible to make extensive use of tropically-adapted breeds in many situations including those in Japan and the United States.

While introduction of tropically-adapted breeds is not always feasible, it is likely that selection for thermotolerance within a breed is possible. Estimates of the heritability of rectal temperature ranged from 0.25 to 0.66 (Finch 1986). Advances in molecular genetics may simplify selection for thermotolerance. Genetic markers for thermotolerance have been identified (Hayes *et al.* 2009) as well as single nucleotide polymorphisms in specific genes such as *ATP1A1* (Liu *et al.* 2011) that affect body temperature regulation during HS. The importance of genotype for a single gene has been shown by Dikmen *et al.* (2008) who studied the effect of introduction of the slick gene affecting hair length into Holsteins. Slick-haired Holstein cows regulated body temperature more effectively than wild-type cows under high ambient temperature.

FACILITIES FOR REDUCING HEAT STRESS

Increased uterine temperature both on the day of insemination and the day after had the greatest negative effect on conception (Thatcher 1974). In Florida (Ealy *et al.* 1994), pregnancy rates were higher in cows cooled by both sprinklers and forced ventilation during final maturation of oocytes and early embryonic development (from 2 to 3 days before until 5 to 6 days after AI) compared with those cows exposed to shade only. In Iran (Moghaddam *et al.* 2009), dairy heifers cooled with sprinkler and fan from 2 h before until 2 h after AI experienced lower rectal temperature (38.7C) than those of cows with sprinklers alone (39.2C) or without cooling (39.3C) at the time of AI and had higher pregnancy rate during heat stress, compared with heifers cooled with sprinkler only and heifers without cooling. Although seasonal variation in reproductive function can persist after altering environment through methods such as shade, fans, and sprinklers (Hansen & Arechiga 1999), the magnitude of seasonal effects is reduced. Therefore, cooling during the summer heat season is very important to reduce body temperature and improve reproductive performance in cows and, in some circumstances, heifers.

Cooling can be of reduced effectiveness when air temperatures are very high, because of reduced conductive and convective cooling, or when humidity is high, because of reduced evaporative heat loss. Evaporative cooling may also not be desirable when straw bedding is used because of the possibility that increased humidity can increase the number of bacteria in the straw (Ward *et al.* 2002).

Probably, the most effective cooling systems currently in use are those that couple evaporative cooling with tunnel ventilation or cross ventilation. In these systems, fans draw air through the end (tunnel ventilation) or side wall (cross ventilation) and the air is humidified by high pressure misters over feed bunks and free stalls. Smith *et al.* (2006a, b) reported that evaporative tunnel ventilation caused increased feed intake

(12% over cows housed outside) and milk yield (2.6 kg/cow per day). In Utsunomiya University located in east Japan, a tunnel ventilation system in a small scale stanchion shed (10 lactating dairy cows) improved body condition and lactation performance in the summer heat (Nagao *et al.* 2009). Moreover, further studies are warranted to evaluate the effects of evaporative tunnel and cross ventilation on reproductive performance in cows and heifers in Japan and to evaluate its cost-effectiveness under Japanese conditions.

CONCLUSIONS

Heat stress can have severe effects on productivity of livestock in Japan as well as many other areas of the world. Rectal temperatures greater than 39.0°C and respiration rates greater than 60 per minute indicate cows are undergoing HS sufficient to affect milk yield and fertility. Methods to alleviate effects of HS include ET to improve fertility. Given the growing importance of HS, development of new approaches to combat HS is justified. Advances in genetic technologies make it likely that cattle can be made more resistant to HS without compromising production. It may be beneficial to introduce evaporative tunnel or cross ventilation systems into Japan to cool cows body and reduce body temperature, although study to determine effectiveness of these housing systems is warranted. More research into this question and others will be required to overcome HS.

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Figure 1 Panting and drooling of a dairy cow exposed to heat stress in Florida (Photo courtesy of J.E. P. Santos).